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# Therapeutic Effect of Flavonoids Derived from *Plantago Species*

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*Flavonoids are widely distributed in plants fulfilling many functions including producing yellow or red/blue pigmentation in flowers and protection from attack by microbes and insects. Current research shows for these compounds an anti-allergic, and an anti-cancer activity. The objectives of the research are the investigation of other therapeutic effects of flavonoid fraction separated from alcoholic extract of* Plantago sp.*, as following: sedative, analgesic and anti-microbial activities. The physical-chemical properties of the extract were also investigated. The results obtained indicate the presence of D-apigenin, lutelin, scutellarin, baicalein, nepetin, hispidulin, plantagoside, potassium and microelements in the flavonoids extract. The anti-inflammatory effect (study performed on mice with pletismometric method)) was meaningful after 120 minute at a dose of 400 mg/kg body. The sedative effect (study performed on mice using the writhing test) was meaningful after 30 minutes at 400 mg/kg body. The antimicrobial activities (study performed "*in vitro*" using Kirby Bauer method) reveal the intense bacteriostatic activity against the microorganisms like* Staphylococcus aureus, Pseudomonas aeruginosa *and* Bacillus sp. *This research suggests that plantain may be effective in the treatment of inflammatory and microbial disease but further studies are needed.*

**Keywords** Anti-inflamatory; effect; flavonoidic extract; sedative

## Introduction

Flavonoids are widely distributed in plants fulfilling many functions including producing yellow or red/blue pigmentation in flowers and protection from attack by microbes and insects. Current research shows for these compounds an anti-allergic and an anti-cancer activity [1,2]. There has been considerable interest in

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the flavonoid content of foods and plants since the early 1980s when the studies were demonstrated a relationship between a diet rich in fruits and vegetables and a reduced risk for chronic diseases. Because reduced risk did not correlate with traditional nutrients, attention has focused on many non-nutrient, potentially bioactive compounds, of which the flavonoids constitute one family [2].

Flavonoids are naturally-occurring polyphenolic compounds with a C6-C3-C6 backbone. This group of plant pigments which are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine can be chemically subdivided into six structural categories: flavones, flavonols, flavanones, flavanonols, flavan-3-ols (catechins), and anthocyanidins. These compounds (aglycones) are commonly glycosylated (at one or more sites with a variety of sugars) and may also be alkoxylated or esterified. As a result, over 5000 different flavonoids have been identified in plant materials [3,4] (Fig. 1, Table 1).



## Experimental

The objectives of this research are the investigation of other therapeutic effects of flavonoid fraction separated from alcoholic extract of *Plantago sp.*, respectively anti-inflammatory and analgesic activity. Biological characteristics regarding the anti-inflammatory and analgesic effects were observed for "*in vivo*" tests on mice, using the pletismometric and hot-plate method.

**Studies regarding anti-inflammatory effect by pletismometric method** were performed using 2 groups of 6 Wistar rats, one witness group (W) and one test group (F). Flavonoidic extract was administrated intraperitoneal (i.p.) as alcoholic solution (20% ethanol in physiological ser) with a dose of 400 mg flavonoidic extract/kg body. As inflammatory reagent, 1% kaolin solution was used.

**Analgesic effect of flavonoidic extract using hot-plate method** was determined using 4 groups of 10 rats (2 test groups and 2 witness groups). The rats from the test groups was i.p. injected with 400 mg flavonoidic extract/kg body, extract administrated as alcoholic solution which contain 4% flavonoidic extract. Alcoholic solution contains 20 g ethanol in 100 g physiologic ser. The tests were performed at 60 and 120 minute respectively. The animals from the witness groups was intraperitoneal (i.p.) injected with a 0.1 mL ethanol/kg body (ethanol concentration was 20%).

**Diffusive Kirby Bauer method was applied for antimicrobial activity** using the following pathogenic microorganisms: *Staphyloccocus aureus, Pseudomonas aeruginosa* and *Bacilus sp.* Antibacterial activity was tested using paper dishes impregnated in 50% solution of flavonoidic extract (F) in comparison with common antibiotics.

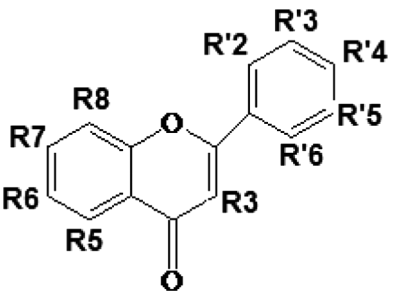


**Figure 1.** General structure of flavonoidic compound.

**Table 1.** Flavonoidic compound contained in *Plantago sp.*

| Flavonoidic compound | R3 | R5 | R6 | R7 | R8 | R2′ | R3′ | R4′ | R5′ | R6′ | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Scutellarein | H | OH | OH | OH | H | H | H | OH | H | H | [3,4] |
| Apigenin 7-glucozid | H | OH | H | OGlc | H | H | H | OH | H | H | [5] |
| Baicalein | H | OH | OH | OH | H | H | H | H | H | H | [3] |
| Hispidulin | H | OH | OMe | OH | H | H | H | OH | H | H | [4] |
| Hispidulin 7-glucuronid | H | OH | OMe | OGlcA | H | H | H | OH | H | H | [5] |
| Homoplantaginin | H | OH | OMe | OGlc | H | H | H | OH | H | H | [5] |
| Luteolin 7-glucozid | H | OH | H | OGlc | H | H | OH | OH | H | H | [5] |
| Luteolin 7-diglucozid | H | OH | H | OGlc-OGlc | H | H | OH | OH | H | H | [5] |
| Luteolin 6-hidroxi-4′-metoxi-7-galactozid | H | OH | OH | OGal | H | H | OH | OMe | H | H | [5] |
| Nepetin 7-glucozid | H | OH | OMe | OGlc | H | H | OH | OH | H | H | [5] |
| Plantaginin | H | OH | OH | OGlc | H | H | H | OH | H | H | [5] |

**Table 2.** Anti-inflamatory effect of flavonoidic extract

| Witness | T1 = 90′ F.V., $cm^3$ | T2 = T1 + 30′ F.V., $cm^3$ | T3 = T2 + 30′ F.V., $cm^3$ | T4 = T3 + 30′ F.V., $cm^3$ |
|---|---|---|---|---|
| W1 | 24 | 27 | 28 | 27 |
| W2 | 22 | 26 | 22 | 23 |
| W3 | 25 | 27 | 27 | 26 |
| W4 | 24 | 23 | 23 | 21 |
| W5 | 20 | 22 | 24 | 24 |
| W6 | 24 | 25 | 26 | 26 |
| Average W (1–6) | 23.1666 | 25 | 25 | 24.5 |
| SD | 1.8348 | 2.1976 | 2.3664 | 2.2583 |
| F | T1 = T0 + 60′ | T2 = T1 + 30′ | T3 = T2 + 30′ | T4 = T2 + 30′ |
| F1 | 16 | 27 | 28 | 26 |
| F2 | 26 | 27 | 24 | 20 |
| F3 | 27 | 24 | 25 | 22 |
| F4 | 24 | 19 | 23 | 17 |
| F5 | 19 | 23 | 24 | 22 |
| F6 | 23 | 29 | 23 | 22 |
| Average F (1–6) | 22.5 | 24.8333 | 24.5 | 21.5 |
| SD | 4.2308 | 3.6009 | 1.8708 | 2.9495 |
| *p*-value | 0.8945 | | 0.5176 | 0.0021 |

SD = standard deviation; *p* = statistic significance treated lot/whitness; F.V. = foot volume.

**Table 3.** Analgesic effect of flavonoidic extract, at 60 minute

| Crt. No. Parameter | Witness (60 minute) | | Flavonoidic extract 400 mg/kg body (60 minute) | |
|---|---|---|---|---|
| | Sleek | Leap | Sleek | Leap |
| 1 | 8.47 | 104.72 | 12.13 | 111.44 |
| 2 | 6.34 | 155 | 6.88 | 96.31 |
| 3 | 8.97 | 73.8 | 5.25 | 109.09 |
| 4 | 8.5 | 78.97 | 9.25 | 104.5 |
| 5 | 7.03 | 180 | 6.81 | 144.37 |
| 6 | 6.03 | 95.66 | 7.63 | 115.31 |
| 7 | 9.28 | 97.13 | 9.19 | 142.84 |
| 8 | 5.39 | 89.62 | 10.15 | 60.94 |
| 9 | 3.13 | 97.19 | 5.75 | 165.82 |
| 10 | 4.13 | 58.12 | 11.18 | 135.81 |
| Average (1–10) | 6.727 | 103.021 | 8.422 | 118.643 |
| SD | 2.1062 | 37.0905 | 2.3195 | 29.7217 |
| *p*-value | | | 0.0521 | 0.1561 |

**Table 4.** Analgesic effect of flavonoidic extract, at 120 minute

| Crt. No. Parameter | Witness (120 minute) | | Flavonoidic extract 400 mg/kg body (120 minutes) | |
|---|---|---|---|---|
| | Sleek | Leap | Sleek | Leap |
| 1 | 9.35 | 85.53 | 10.15 | 88.09 |
| 2 | 10.63 | 106.37 | 11.16 | 124.28 |
| 3 | 7 | 106.22 | 10.59 | 107.9 |
| 4 | 9.56 | 105.16 | 7.21 | 106.32 |
| 5 | 6.81 | 91.78 | 9.94 | 104.69 |
| 6 | 10.06 | 112.5 | 8.94 | 120.75 |
| 7 | 5.12 | 79.41 | 7.44 | 114.57 |
| 8 | 11.63 | 109.29 | 9 | 180 |
| 9 | 14.63 | 124 | 9.47 | 131.5 |
| 10 | 8.97 | 124.09 | 10.31 | 91.72 |
| Average (1–10) | 9.376 | 104.435 | 9.421 | 116.982 |
| SD | 2.6929 | 14.9405 | 1.2998 | 25.9818 |
| *p*-value | | | 0.4812 | 0.1010 |

**Physical and chemical analyses of the solid extracts with flavonoids** have been performed by energy dispersive X-ray fluorescence using a spectrometer type PW 4025 MiniPal, atomic emission with inductively coupled plasma using a spectrometer type ICP-AES Varian Liberty 110, CHNS/O analyser type Perkin Elmer Series II, 2400, infrared spectra using a FT-IR spectrophotometer type Spectrum GX Perkin Elmer with accessories: DRIFT (Diffuse Reflectance Infrared Fourier Transform) and ATR (Attenuated Total Reflectance), thermo-gravimetric and differential thermal analysis using a Mettler-Toledo thermogravimetric analyzer type TGA/SDTA851$^e$ and Differential scanning *calorimeter type* DSC 823$^e$ Metter Toledo.

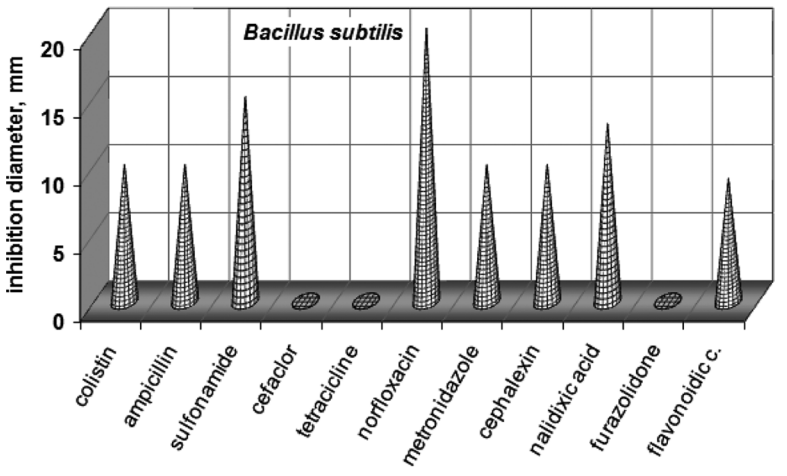


**Figure 2.** Effect of flavonoidic extract against *Bacillus subtilis*.

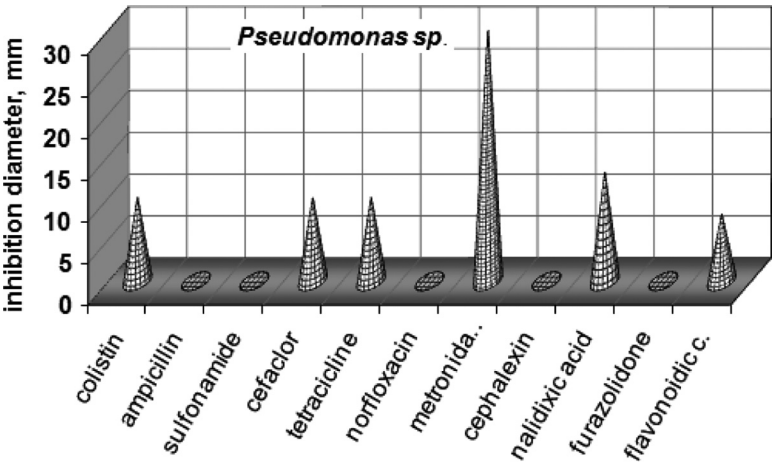


**Figure 3.** Effect of flavonoidic extract against *Pseudomonas aeruginosa*.

## Results and Discussion

### *Anti-Inflammatory Effect of Flavonoid Extract*

Studies performed in order to establish the anti-inflammatory effect of flavonoidic extract derived from *Plantago sp.* show an effect at 120 minutes after administration of 1% kaolin suspension, whereas the anti-inflamatory effect appears after 180 mins when a dose of 400 mg flavonoidic extract/kg body was i.p. administrated (results statistic significantly, $p < 0.05$).

In this case the obtained results indicate an anti-inflammatory effect of flavonoidic extract, slowly install, and possibly for long time (Table 2). Average volume of the foot mice was 24.5 $cm^3$ in the witness case and 21.5 $cm^3$ when flavonoidic extract was administrated. Difference between the two lots was statistic significantly ($p < 0.05$).

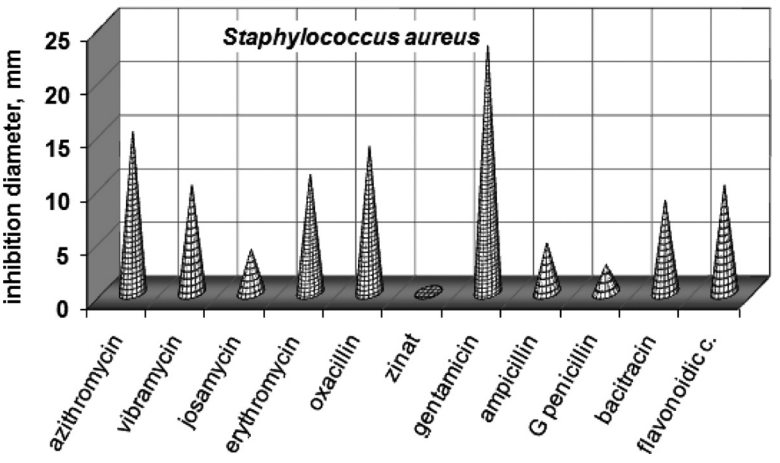


**Figure 4.** Effect of flavonoidic extract against *Staphylococcus aureus*.

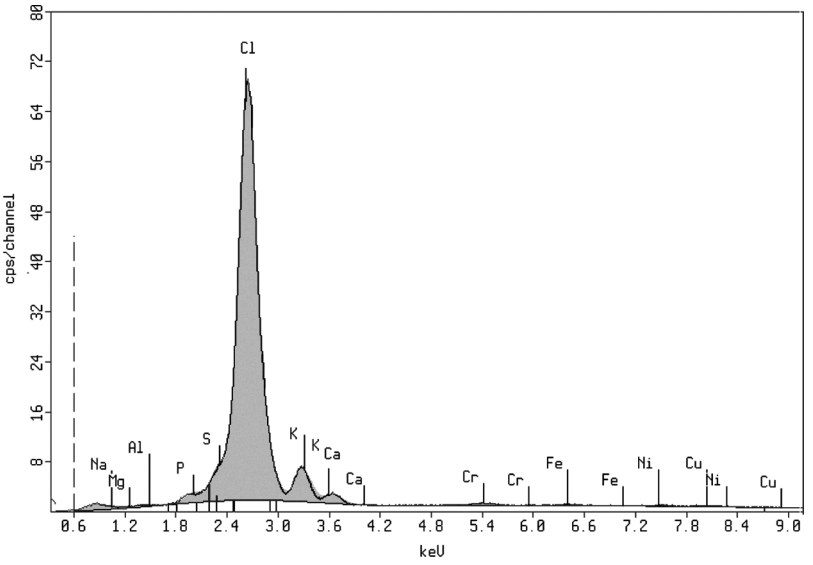


**Figure 5.** Qualitative analysis of flavonoidic extract derived from *Plantago sp.*

## Analgesic Effect of Flavonoid Extract

Flavonoidic extract give significantly results for sleek parameter after 60 minutes at 400 mg/kg body dose administration. Results obtaining for leap parameters is not significantly for 60 and 120 minutes before testing (Tables 3 and 4). For this extract, experimental results reveal the increase of pain perception level at 60 minute after i.p. administration of the dose of 400 mg extract/kg body (sleek parameter).

## Antimicrobial Effect of Flavonoidic Extract

Flavonoidic extract (F) have a bacteriostatic effect against all microorganisms tested. In the case of *Bacillus sp.*, (Fig. 2) flavonoidic extract reveal a bactericidal effect in comparison with common antibiotic like: cefaclor, tetracycline and furazolidone.

**Table 5.** Elemental analysis flavonoidic extract obtained from *Plantago sp.*

| Element | % | Element | % |
|---|---|---|---|
| C | 51.14 | Mn | 0.00 |
| H | 5.96 | Fe | 0.009 |
| N | 2.09 | Mg | 0.0129 |
| S | 0.98 | Na | 0.066 |
| Ca | 0.058 | B | 0.072 |
| K | 3.45 | Zn | 0.014 |
| P | 0.00 | Cu | 0.015 |

**Table 6.** Infrared spectra of flavonoidic extract

| Wavenumber, $cm^{-1}$ | Assignment and comment |
|---|---|
| 813 | $\nu$C−H overlapped on ring vibration meta and para disubstituted benzene |
| 1033 | $\nu$C−O−C aromatic ethers [2] |
| 1160 | $\nu$C=C; $\nu$ C−O−C aromatic ethers [2] |
| 1280 | $\nu$C−O−C aromatic ethers [2] |
| 1374 | $\nu CH_3$; identified in asperulosidic acid [2] |
| 1447 | $\nu$C=O ketones from aromatic rings |
| 1517 | $\nu$C=O ketones from aromatic rings |
| 1605 | $\nu$C=O ketones from aromatic rings (band indentified in Forythoside B ($C_{34}H_{44}O_{19}$) [2] |
| 1695 | $\nu$C=O, aryl ketone; carboxylic acid aromatic/unsaturated flavone (band indentified in feretoside, lamiide [2]) |
| 3407 | $\nu$O−H (phenols); $\nu$ N−H (amines) |

**Table 7.** Thermal analysis of flavonoidic extract

| Temperature range, °C | Thermic effect | Loss, mass % | Comments |
|---|---|---|---|
| 25–100 | 75°C; $\Delta H > 0$ | 4.70 | Deshidratation |
| 100–200 | 173°C; $\Delta H < 0$ | 5.49 | Organic decomposition |
| | 196°C; $\Delta H < 0$ | | |
| 200–400 | 231°C; $\Delta H < 0$ | 43.52 | |
| 400–600 | 441°C; $\Delta H > 0$ | 93.18 | |
| | 572°C; $\Delta H < 0$ | | |
| 600–1000 | $\Delta H > 0$ | 90.39 | |
| Total loss (25–1000°C) | | 99.66 | Organic decomposition |

For *Pseudomonas aeruginosa*, the flavonoidic extract shown the same bacteriostatic effect (Fig. 3) in comparison with ampicillin, sulfonamide, norfloxacin, cephalexin and furazolidone.

Effect of flavonoidic extract against *Staphyloccocus aureus* reveal again a bacteriostatic effect in comparison with josamycin, zinat, ampicillin, penicillin (lactam antibiotics) (Fig. 4).[1] Development inhibition of the three microorganism in the presence of flavonoidic extract reveal the powerfully bacteriostatic effect, probably due to insufficient purification of them.

## Physical-Chemical Analysis of Flavonoidic Extract

Qualitative and quantitative analyses of flavonoidic extract indicate the presence of C, H, N, K (Fig. 5 and Table 5) in large amounts, and traces of Mn, Fe, Mg, Na, B

[1]For the active substance from these antibiotic see the article N. Radu *et al.* Therapeutic effect of polysaccharides from *Plantago sp.* in this number.

and Zn. Infrared spectra reveal the presence of flavonoidic compound (Table 6) due to presence of specific bands from aromatic ring and unsaturated flavones respectively.

Thermal analysis indicate the stability of bioproduct in the range (25–75)°C (Table 7); after 75°, biomaterial was decomposed, in the first stage by release inter or intramolecular water and after that by organic material decomposition up to 600°C, when almost all biomaterials are transformed in $CO_x$ and $H_2O$ (99,66% loss weight)

## Conclusions

The anti-inflamatory effect (study performed on mice with pletismometric method) was meaningful after 120 minute at a dose of 400 mg/kg body. The analgesic effect of flavonoid extracts is not concluded because we obtained very good results for sleek parameter but insignificantly results for leap parameters.

Qualitative and quantitative analyses revealed the presence of C, H, N, and some microelements in the flavonoidic extracts as well as the presence of flavonoidic compounds and the thermolability of this extract. Flavonoidic extract have a better bacteriostatic effect against *Bacillus sp., Pseudomonas aeruginosa and Staphyloccocus aureus* comparing with common antibiotic. This research suggests that this plantain extract may be effective in the treatment of inflammatory disease but more detailed studies are still necessary in order to elucidate if the flavonoidic extract has or not anti inflammatory effect.

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